Radiation Physics and Chemistry 96 (2014) 115–119

Contents lists available at ScienceDirect

Radiation Physics and Chemistry

journal homepage: www.elsevier.com/locate/radphyschem

Synthesis of hybrid polymer networks of irradiated chitosan/poly(vinyl alcohol) for biomedical applications

Atif Islam [a,b], Tariq Yasin [a,*], Ihtesham ur Rehman [c]

[a] Department of Metallurgy and Materials Engineering, Pakistan Institute of Engineering and Applied Sciences, Nilore 45650, Islamabad, Pakistan
[b] Department of Polymer Engineering and Technology, University of the Punjab, 54590, Lahore, Pakistan
[c] Department of Materials Science and Engineering, The Kroto Research Institute, North Campus, University of Sheffield, Broad Lane, Sheffield S3 7HQ, UK

HIGHLIGHTS

- Biocompatible HPN are prepared from irradiated chitosan.
- XRD showed the decrease in crystallinity of the HPN with increase in dose.
- Lowering of contact angle with increase in dose showed increased hydrophilicity.
- Cytotoxicity results showed nontoxicity of HPN and viability of the cells.

ARTICLE INFO

Article history:
Received 9 April 2013
Accepted 18 September 2013
Available online 27 September 2013

Keywords:
Chitosan
Radiation
Chain scission
Biomedical applications

ABSTRACT

Hybrid polymer network (HPN) of chitosan (CS) with poly(vinyl alcohol) (PVA) was prepared by using radiation degraded chitosan. The chemical structure of chitosan promoted chain scission reactions upon irradiation which lowered its molecular weight and also changed its hydrophilic balance. The effect of molecular weight and hydrophilicity of irradiated chitosan on structural, thermal and surface properties of the HPN were studied. The increased hydrophilicity of irradiated chitosan lowered the crystallinity of the HPN. The endothermic peak was shifted towards higher temperatures in HPN having irradiated chitosan. The decreased value of contact angle with increasing dose, further confirmed the increased hydrophilicity of the HPN. The cytotoxicity results of HPN showed the viability of human fibroblast cells and their non-toxic nature making it suitable for tissue engineering and other biomedical applications.

1. Introduction

Chitosan is a naturally occurring polymer, manufactured by deacetylation of chitin extracted from fungi and crustacean shells. It has different degrees of deacetylation and molecular weight depending upon the source and the processing technique (Duy et al., 2011; Islam and Yasin, 2012). Chitosan is cationic polysaccharides which forms electrostatic interactions with cells having negatively charged surface. Chitosan can be fabricated into different shapes and forms with good hydrophilicity, cell adhesive and proliferation properties (Hsieh et al., 2006; Ma et al., 2010; Islam et al., 2012).

Chitosan has molecular weight ranging from 300 to 1000 kDa and the degree of deacetylation is from 30% to 99% (Madihally and Matthew, 1999). The molecular weight of chitosan is lowered by different methods and the use of radiation for controlling the molecular weight is safe, environmental friendly, free from chemicals and initiator residues (Nho et al., 2005; Wasikiewicz et al., 2005; Dubey et al., 2009). For biomedical applications, the stable structure of chitosan is obtained by crosslinking its chains which retain water or bioactive compounds without dissolution. The crosslinking of chitosan in the presence of other polymers shows variety of behaviors which can be divided into three categories: as semi- or full-interpenetrating polymer networks, chitosan crosslinked with itself and hybrid polymer networks (Berger et al., 2004; Rodrigues et al., 2007). These approaches for its modification and utilization in biomedical applications are extensively explored (Shu, 2004; Chung and Park, 2007; Costa-Pinto et al., 2011). The HPN of chitosan with other polymers are used for tissue incorporation and osteoconductive applications in tissue engineering. The developed materials are biodegradable, biocompatible, bioadhesive and have better mechanical properties (Hutmacher, 2000; Sarasam and Madihally, 2005; Jiang et al., 2006; Yu et al., 2006; Chan and Leong, 2008; Yang et al., 2008; Carletti et al., 2011).

In this work, radiation degraded chitosan is used to prepare HPN using the dissolution casting method to investigate the effect

* Corresponding author. Fax: +92 51 2208070.
E-mail address: yasintariq@yahoo.com (T. Yasin).

0969-806X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.radphyschem.2013.09.009

of molecular weight of chitosan on structural, thermal and surface properties of HPN. The in-vitro cell viability of the HPN is also carried out by cytotoxicity analysis with different human fibroblast cell lines.

2. Experimental and methods

Chitosan was extracted from crab shell using standard methods. Its degree of deacetylation was 75%. PVA (M_w: 85,000–90,000), acetic acid, industrial methylated spirit (IMS) and methylthiazolydiphenyl-tetrazolium bromide (MTT) were purchased from Sigma Aldrich (Milwaukee, WI) and used as received.

2.1. Gamma irradiation

Irradiation of chitosan was carried out at Pakistan Radiation Services using ^{60}Co gamma irradiator (Model JS-7900, IR-148, ATCOP) in air atmosphere at a dose rate of 1.02 kGy/h. The chitosan was subjected to different doses of gamma radiation ranging from 25 to 100 kGy.

The chitosan was dissolved in acetic acid (0.5 M) and PVA was dissolved in deionized water. The weight ratio of CS and PVA was 1:4. Both the solutions were mixed at room temperature and stirred for 2 h. The solution was poured into petri dishes at room temperature to get HPN. The codes of the HPN are given in Table 1.

2.2. FTIR spectroscopy

FTIR spectroscopy was done using FTIR spectrophotometer (Nicolet, 6700) from Thermo Electron Corporation, USA. Attenuated total reflectance mode with diamond crystal was used in the range of 4000–400 cm^{-1} at a resolution of 6.0 cm^{-1} and average of 200 scans was reported.

2.3. XRD analysis

The XRD analysis was carried out on STOE STADI P power diffractometer with (Cu) $K\alpha_1$ radiation (1.54 Å) at scanning rate of 0.2 °/min from 5° to 60° (2θ). The peakfit program was used for peak deconvolution and crystallinity (%) was calculated by area under the curve of characteristics peak.

2.4. Differential scanning calorimetry

The differential scanning calorimetry was performed using Perkin-Elmer DSC 7 differential scanning calorimeter. The samples were scanned from 40 to 200 °C at a heating rate of 10 °C/min under nitrogen atmosphere.

2.5. Contact angle measurement

The contact angle of the HPN was evaluated using the contact angle Goniometer Model 100-00 (220) (Ramé-hart Instrument company). A small piece of HPN was placed on the sample stage of the equipment. A uniform drop (5 μL) of distilled water was placed on the surface of the sample. The contact angle was estimated until the drop was vanished. Each measurement was taken for three times and the average value was reported.

Table 1
Sample codes and molecular weight of chitosan.

Sample codes	HPNC	HPN25	HPN50	HPN75	HPN100
Dose (kGy)	0	25	50	75	100
Molecular weight (g/mol) ($\times 10^4$)	22.00	11.50	8.97	7.17	5.53

The ratio of CS and PVA in the HPN is 1:4.

2.6. Cytotoxicity analysis

The MTT assay was performed for cytotoxicity analysis of the HPN. Human fibroblast cells lines (F121, F192 and F84 for the indirect method) and human dermal fibroblast cell (F121 for the direct method) were provided in Dulbecco's Modified Eagle Medium (DMEM) in tissue culture plastic flasks and incubated at 37 °C in atmosphere containing 5% CO_2. The HPN was placed in well plates having cells in DMEM (1 mL). The cells density was 5000 cell/well with 80% confluency. The samples were washed with IMS and phosphate buffer saline (PBS) and placed in the well plates for the direct method. In the indirect method, samples were placed in transfer filters along with the cells. DMEM media (300 μL) was added in each well plate and incubated for 24 h. The well plates were taken out from the incubator, eliminated all the media, plates were washed with PBS and added 1 mL of MTT solution (0.5 mg/mL in PBS) was added in each well. After incubation for 1 h, the MTT solution was removed and 2-ethoxy methanol (300 μL) was added in each well for the elution of purple formazan. 150 μL of this solution was shifted to the 96-well plate. The optical density was measured using microplate analyzer (Spectrophotometer Biotek Instruments) at 562 nm. The positive and negative controls were also prepared for comparison purpose.

3. Results and discussion

The viscosity average molecular weight of chitosan is 22.0×10^4 g/mol. The irradiation of chitosan in air affects its molecular weight and the viscosity average molecular weight of irradiated chitosan used in this study is given in Table 1. This table shows a sharp decrease in molecular weight of chitosan at 25 kGy and lowest molecular weight of 5.53×10^4 g/mol is obtained at 100 kGy irradiated sample. The gamma radiation dissociates the C–H and C–OH bonds present in chitosan which generates macroradical along with hydrogen and hydroxyl radicals in the sample. The reaction of hydrogen and hydroxyl radicals with polymer chains produced more macroradical which undergoes variety of reactions such as: chain scission, hydrogen transfer, disproportionation, inter- and intra-molecular recombinations. The result of these reactions is the breakage of the polymer backbone which lowers the molecular weight of chitosan.

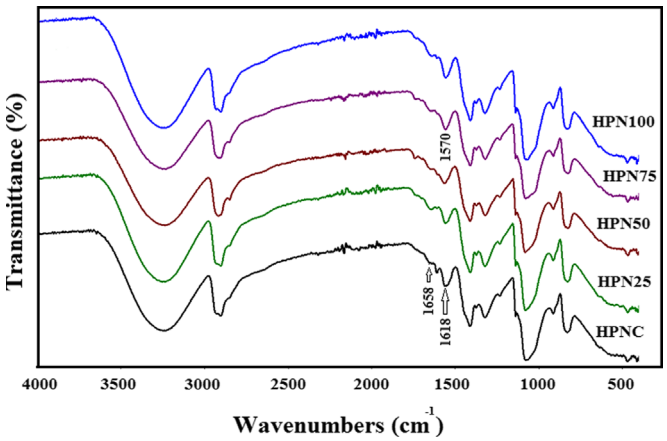

Fig. 1. FTIR spectra of HPN containing irradiated chitosan in the range of 4000–400 cm^{-1}.